UPDATED STUDY DESIGN [1]
TOTAL EXPOSURE PILOT STUDY

Study Objective:

The *objective* of the present pilot study is to establish the validity of the design concepts (e.g., collection, feasibility and precision of analytical methods for biomarkers, sample collection, handling, and stability, data acquisition by questionnaires and diary) to be used in the subsequent Total Exposure Study. In particular the study should determine the intra- and inter-individual variability of each estimate of smoke constituent uptake as a basis for the design of the Total Exposure Study. The results and evaluations of the pilot study should be complete and made available to Philip Morris within the year 2001.

Study population: [2]

The study population will comprise of adult smokers currently smoking cigarette products with 3.0-6.9 mg tar (FTC) delivery per cigarette (some called "ultralight") and non-tobacco-users. This tar band contains approximately 10 % of the U.S. smoking population. The tar delivery as reported by the TITL, 1999, of the preferred brand style reported by the adult smokers will be used to allocate adult smokers to the smoker group. There is no selection for cigarette manufacturers, cigarette design and composition (e.g. mentholated or non-mentholated). Adult current smokers are defined by reporting to smoke regularly at least 1 cigarette of an eligible brand style per day[3] for at least 1 year.

Non-tobacco-users are defined as adult subjects not smoking at all for the last year or using any nicotine-containing products such as snuff, chewing tobacco, patches, and sprays during and 3 months before the sample collections for this study. Their reported absence of active nicotine uptake will be verified by determination of urinary cotinine. Persons with urinary cotinine exceeding 50 ug/l will be excluded from this group.

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[1] The design outlined here represents the design of the pilot study. The current design draft for the Total Exposure Study is added as an Appendix for reference purposes. We do invite you to suggest modifications based on your experience in clinical/population studies, understanding of the objectives of the study and knowledge about the scientific aspects of the analytical goals of the study.

[2] The study population in the pilot study does not need to be representative of the age, gender, ethnic, and socioeconomic distribution of the U.S. adult cigarette smoking population, but should represent a randomly selected group of smokers.

[3] Not a mean over a week or month ('chippers' excluded)

Sample size and grouping:

Sixty adult 3.0-6.9 mg-smokers, and 60 adult non-tobacco-users (control group) each. Each group will contain 30 males and 30 females. The total number of subjects for the pilot study will therefore be 120.

Dropouts will be replaced.

Subject exclusion criteria:

Subjects who report the following characteristics will be excluded from the test sample:

- Smoke a cigarette brand with tar delivery per cigarette different than the specified range (3.0-6.9 mg/cig.);
- Have switched to a new brand during the last 3 months prior to the sample collection;
- Use of other cigarette brand(s) at a level >10% of the consumption of their preferred cigarette brand during the last 3 months before sample collection;
- Use of any nicotine-containing product other than manufactured cigarettes;
- Diseases (reported as diagnosed plus confirmation of healthy status by routine hematology, pulmonary function test Fev_1, and ECG) which could interfere with the measured health effect surrogates such as all currently diagnosed cancers, uncontrolled diabetes, coronary heart disease, uncontrolled hypertension, stroke, heart infarction, bronchitis (acute and chronic), emphysema, asthma, renal dysfunction, hyperlipidemia;
- Pregnant/nursing women;
- Persons of less than 21 years of age (age verification required by copy of government issued identification);
- Employees and first degree relatives of the tobacco industry;
- Employees and first degree relatives of the CRO;
- Subjects exceeding 50 ug cotinine/l urine for the non-tobacco user group;
- Subjects where reported number of cigarettes smoked and butts collected differ by more than 10%;
- Participants in other clinical studies;
- Persons who donated blood or received blood transfusions during the study and during the 3 months prior to enrollment.

Sample collection:

- Venous blood (4 samples per subject, one each at week 1, 2, 3, and 6; 50 ml each);
- Exhalate (Tedlar bag; 4 samples per subject one each at week 1, 2, 3, and 6; 500 ml each);

- 24-h urine (3 24-h samples per subject, one each at weeks 1, 3, and 6; storage below room temperature before transfer to the clinical facility);
- Cigarette butts of all cigarettes smoked during the days of urine sample collection;
- All packs from which smoked cigarettes were drawn during days of sample collection;
- Induced sputum (1 sample per subject, week 3).

Biomarkers selected:

To minimize invasive sampling requirements and in accordance with NRC selection criteria as proposed by Benowitz the following biomarkers for exposure were selected:

BIOMARKER	SAMPLE MATERIAL	SMOKE CONSTITUENT	SMOKE PHASE (b)	SAMPLING WEEK
Acetonitrile	Exhalate	Acetonitrile	GVP	1, 2, 3, 6
Carbon monoxide	Exhalate	Carbon monoxide	GVP	1, 2, 3, 6
Carboxy-hemoglobin	Blood (whole)	Carbon monoxide	GVP	1, 2, 3, 6
Hb adducts of 3- and 4- aminobiphenyl	Blood (10ml, whole)	3- and 4-aminobiphenyl	PP	1, 6
Nicotine and nicotine metabolites (a)	24-hr urine (c) (50 ml)	Nicotine	PP	1, , 3, 6
NNAL and NNAL-glucuronide	24-hr urine (c) (500 ml)	NNK (d)	PP	1, 3
Cadmium	Blood (8 ml, whole)	Cadmium	PP	1, 3, 6

(a) cotinine, 3-hydroxycotinine, nicotine glucuronide, cotinine glucuronide, 3-hydroxycotinine glucuronide,
(b) GVP: gas-vapor phase; PP: particle phase
(c) Results expressed in mass/24h and mass/ mg creatinine
(d) NNK: 4-(methylnitrosamino) –1-(3-pyridyl)-1-butanone

In addition the following biomarkers of susceptibility/potential health effects will be analyzed (preliminary selection) as a basis for future studies (exploratory) in weeks 1, 3 and 6:

BIOMARKER	SAMPLE MATERIAL	RELATED HEALTH ENDPOINT
Thromboxane B2	24-hr urine (a)	Atherosclerosis
8-epi-prostaglandin F2 alpha	24-hr urine (a)	Lipid peroxidation
HDL, LDL	Blood (serum)	Atherosclerosis
Malondialdehyde	Blood (serum)	Oxidative stress
Fibrinogen	Blood (serum)	Cardiovascular disease
C-reactive protein	Blood (plasma)	Tissue injury, inflammation, neoplasm

(a) Results expressed in mass/24h and mass/ mg creatinine

Storage of sample materials for future analyses:

Blood (serum, white blood cells, and red blood cells separately), induced sputum (cells and supernatant separately), and urine samples will be stored at minus 70 degrees centigrade for future (exploratory) studies of biomarkers of health effects relevant to determine reduced harm of cigarette products to adult smokers. Consent will be sought from subjects to allow genotyping of CYP polymorphisms and testing for future biomarkers of exposure, potential health effects, with the proviso that reports of such results will not be shared with subjects nor contain subject identification.

Analytical methods for biomarkers:

Methods with adequate sensitivity, reproducibility, and selectivity will be used. Analytical determinations should be based on intra-laboratory validated methods (e.g., precision runs, linearity of dynamic range) and is encouraged to comply with clinical standards (CLIA) or GLP (compliance will be required in the main study.

Questionnaire evaluations:

The objectives for the questionnaire are:

- To check for exclusion criteria;
- To allow for regression analyses;
- To allow for analyses of 'outliers';
- To evaluate aspects of smoking behavior.

By interviewing all subjects with the help of trained staff and using a structured electronic questionnaire the following information will be collected from all study subjects:

- Demographics and socioeconomic status;
- Health status and history;
- All current medication/supplementation including herbal or vitamin supplements;
- Exposure to cigarette smoke by active smoking based on information on brands smoked (including "tar" and nicotine yield (FTC listing), if printed on pack, mentholation, filter type, and cigarette length) for a specified timeframe, daily tobacco consumption;
- Smoking characteristics (e.g., inhalation/puffing);
- Use of non-tobacco nicotine products;
- Fagerstrom questions (6);
- Exposure to ETS (strength and duration);
- Occupational exposures (in particular those that could interfere with the selected biomarkers);
- Diet characteristics including alcohol consumption;
- Hobbies;
- Home heating systems;
- Exposure to car exhaust;
- Start of last menstrual period (women);
- Physical activity.

Diary:

A diary will be requested for all subjects to report during the 2 days prior to and during the day of urine sample collection:

- Smoking activities;
- Exposure to ETS;
- Diet (eating and drinking).

Other analyses:

Five percent of the collected samples for Hb-adducts, nicotine metabolites, NNK metabolites and cadmium will be shipped to reference laboratories (e.g., INBIFO, Cologne) for independent **verification analyses**. All of these blood samples will require certification for absence of HIV and Hepatitis B and C virus.

Each participant will require **phenotyping for CYP1A2 and NAT-2** activity using caffeine for the test.

In addition to the biomarkers of exposure and susceptibility/potential health effects (see above) **creatinine in urine** will be determined.

The filters from all smoked cigarettes of each participant in week 1 and 6 will be shipped to Dr. L. Kozlowski for **pattern recognition analyses**.

The filters from all smoked cigarettes of each participant in weeks 2 and 3 will be shipped to INBIFO for **analyses of filter retained nicotine**.

Study organization:

A *contract research organization (CRO)* will be engaged to manage, execute or subcontract and oversee the following tasks:

- Advise Philip Morris on potential modification of its study design in the context of the objectives of the pilot study and the Total Exposure Study.
- Cooperate with an external primary investigator (recognized external expert in an area relevant for this study, experienced in managing human studies with bio-analytical focus)
- Establishment (e.g., design/print/ship/review) of a detailed study protocol;
- Preparation/Participation of meeting with Philip Morris and collaborators/ subcontractors;
- Ethical committee consent;
- Sample population selection and recruitment;
- Payment administration to subjects and subcontractors;
- Informed Consent declarations (e.g., preparation/execution/documentation) by study subjects;
- Individual subject documentation (e.g., design/print/translation/shipment/review of case report form (CRF));
- Training of interviewers;
- Questionnaire and diaries evaluation;
- Site identification/selection;
- Development of randomized schedule;
- Collection of biological samples;
- Transportation of collected samples to analytical laboratory and sample storage;
- Selection of analytical laboratory(ies) (requires consent by Philip Morris);
- Biomarker analyses;

- Ensuring subject compliance with study protocol and sample collection protocols;
- Validation of reported smoking (number of cigarettes and brand) against collected cigarette butts;
- Full regulatory compliance including GCP (FDA and ICH regulations) and GLP compliance (if available) and collection/review of regulatory documents (e.g. local IRBs, central IRB);
- Data management planning meeting;
- Development of data management manual;
- Design of database;
- Development of program data edit specifications;
- Review of questionnaires/diaries against exclusion criteria;
- CRF tracking by specified method;
- Data entry/editing/verification;
- Generation/resolution of edits/queries;
- Imaging;
- Data coding;
- Incorporation of analytical data into database;
- Progress reporting;
- Database transfer (e.g., SAS);
- Development of statistical analysis plan;
- Data review meeting;
- Statistical evaluations;
- Final statistical report;
- Writing/editing/shipment/copying of final integrated report(s).

The *scientific review board* (if nominated) will:

- Advise Philip Morris on the design, execution, evaluation, and reporting of the study
- Contribute to the development of the protocol for the pilot study;
- Ensure the study quality.

Philip Morris will:

- Provide a study design for the pilot and Total Exposure Study;
- Provide information about all U.S. cigarette brands for the 3.0 to 6.9 mg tar segment;
- Select and contract with scientific reviewers ;
- Select additional quality assurance oversight functions;
- Provide adequate funding;
- Provide other study materials and support as required and agreed.

END OF DESIGN

Appendix

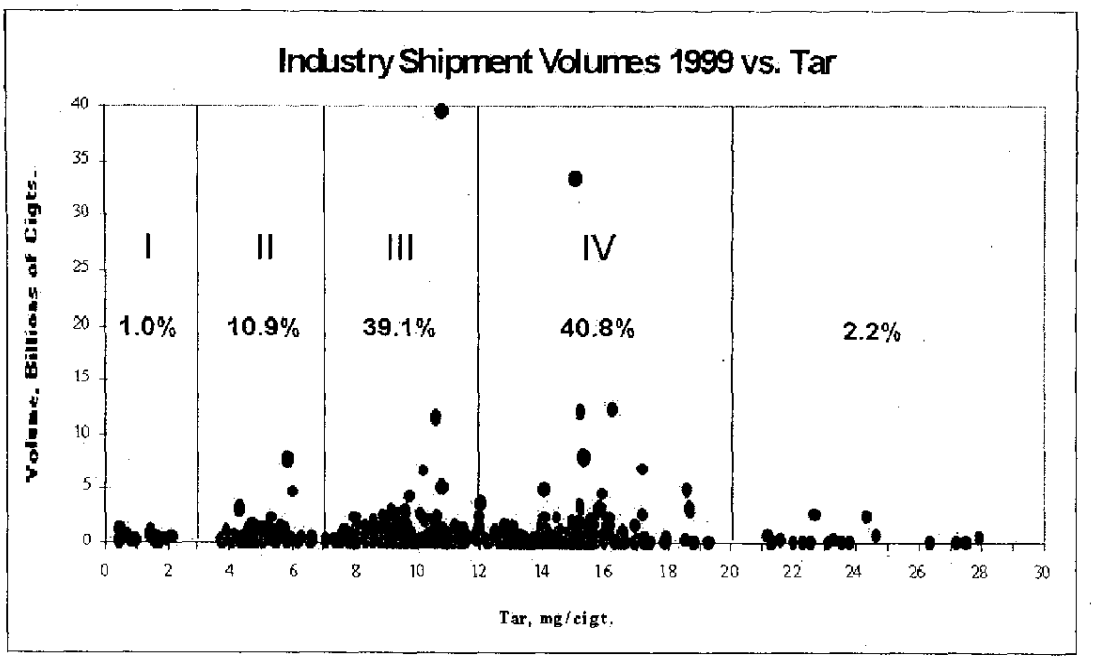
The Total Exposure Study (TES; main study) is intended to provide a 'baseline' estimate of the exposure of the U.S. population of adult cigarette smokers to selected smoke constituents in both the gas/vapor and the particle phase of cigarette smoke. It should provide reference information and methodological insights for a subsequent series of studies in adult smokers. These later studies could investigate the effect of changes of cigarette design, which are aimed at harm reduction, on exposure to smoke constituents.

The *primary objective* of the TES is to determine the exposure of the U. S. population of adult cigarette smokers to whole cigarette smoke based on suitable biomarker(s) and to publish results by 12/31/2001. The *secondary objective* is to investigate whether the smoke exposure of U.S. adult cigarette smokers differs for 4 segments of FTC tar delivery covering the range from 1 to 20 mg tar. In addition, selected biomarkers of potential health effects/susceptibility will be explored for use in population studies, and biological samples will be collected and stored for later determinations of biomarkers of health effects relevant to potentially reduced harm of cigarette products to adult smokers.

The TES seeks to estimate the exposure of populations and to test a research hypothesis. The population estimate is the frequency distribution of exposure to specific smoke constituents (as measured by biomarkers) for U.S. adult smokers and non-smokers. The estimates obtained for non-smokers will provide a measure of the background level of exposure. The research hypothesis is to test if the smoke exposure of adult smokers differs by tar groupings of the cigarettes they regularly smoke i.e., <3.0, 3.0-6.9, 7.0-11.9, and 12.0-20.0 mg tar (FTC measures) per cigarette for each smoke constituent.

The TES has primarily a cross-sectional design but will contain a follow-up of those participants after 1 year, who have decided to switch to another preferred brand.

Marketshare in different tar segments:



Ref.: Tobacco Institute Testing Laboratory

Demographics of tar group '3.0 to 6.9':

	Distribution (%)				
	Total	Full Flavor (13+mg)	Flavor Low (7-12mg)	Ultra Low (3-6mg)	Super Low/Lowest (0-2mg)
Total	100.0	42.5	43.7	11.1	1.4
Gender					
Male	51.0	59.7	46.7	36.2	29.3
Female	49.0	40.3	53.3	63.8	70.7
Age					
18-24	15.7	17.6	16.4	8.9	0.9
25-34	21.0	21.3	22.8	16.0	2.7
35-44	25.7	26.1	25.8	25.8	17.9
45-54	19.8	19.2	18.8	24.6	27.3
55-64	10.5	9.8	9.7	11.7	23.9
65+	7.2	6.0	6.5	10.9	27.3
Race					
White	88.7	72.9	85.5	89.6	91.9
Black	8.6	14.8	4.4	2.9	2.1
Hispanic	4.0	5.1	3.6	2.0	0.8
Asian	1.2	1.0	1.4	1.0	0.9
Native American	1.4	1.7	1.3	1.1	0.6
Mixed	0.9	1.2	0.9	0.4	0.6
Other	0.8	0.8	0.7	0.7	0.8
Education					
No College	54.0	62.0	49.9	42.5	36.2
Any College	43.6	35.4	47.8	55.6	61.9
Income ($)					
< 10,000	5.6	7.2	4.6	3.5	2.6
10,000 - 19,999	13.2	15.6	11.8	9.4	7.5
20,000 - 29,999	14.9	15.9	14.5	13.3	8.4
30,000 - 49,999	27.0	25.5	28.5	27.4	23.0
>= 50,000	22.1	17.9	24.0	28.8	35.0

5